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THE HYDRODYNAMICS OF MICTURITION

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The following is a report of investigations made to compare the hydrodynamics of micturition under normal and pathological conditions, with the aim of revealing disparities, and thus making possible a more functionally based classification and improved diagnostics of urethral and vesical disorders.

The problem has acquired current interest through the recently published technique of percutaneous catheterisation of the bladder, which has made measurement of the intravesical pressure during micturition a relatively easy procedure. (Sandøe, Bryndorf & Gertz, 1959).

NOMENCLATURE

The term *micturition pressure* indicates the intravesical pressure during micturition. The *velocity* stands for the mean velocity of fluid particles at the urethral orifice while the *rate of flow* or simply *flow* states the volume of urine discharged per unit of time. Together these factors are referred to as the *hydrodynamic parameters of the micturition* and are designated in cm of water, cm/s and cm^3/s respectively.

THEORETICAL CONSIDERATIONS

The dull colour and irregular surface of the urinary stream show that the urinary flow is turbulent (Schwartz & Brenner 1922, Morales & Romanus 1952). Consequently the intravesical pressure must be proportionate to the square of the urinary flow. By representing the micturition pressure with p, the urinary velocity with v and the flow with Q this formula is given: 1) $p = K_1 \cdot v^2$ and $p = K_2 \cdot Q^2$ where K_1 and K_2 are constants depending on the dimensions of the tube. It is therefore a non sequitur to say that the urethra has a constant resistance of flow, because the resistance will increase with the rise in pressure.

A comparison of the urethral conductibility in different individuals therefore necessitates a determination of the resistance, or — better — the urinary velocity and rate of flow at one and same pressure of micturition in each individual subjected to examination. For this purpose we have used the eponyms *standard micturition pressure* and *standard values of velocity and flow*. The standard pressure is established at 64 cm of water*). The standard values represent the urinary flow and velocity at a voiding pressure equal to the standard pressure, and are computed from concurrent determinations of the three hydrodynamic parameters according to the following principles.

When using the symbols p_m, v_m and Q_m for concurrent determined values of pressure, velocity and flow respectively, p_{st} for the definetely established standard pressure and v_{st} and Q_{st} for the corresponding standard values of velocity and flow, formula (1) now reads:

$$p_m = K_1 \cdot v^2_m,\ p_{st} = K_1 \cdot v^2_{st}, \text{ and}$$

$$p_m = K_2 \cdot Q^2_m \text{ and } p_{st} = K_2 \cdot Q^2_{st}$$

or

$$v_{st} = v_m \cdot \sqrt{\frac{p_{st}}{p_m}}$$

$$\text{and } Q_{st} = Q_m \sqrt{\frac{p_{st}}{p_m}}$$

Put into words this means that the standard values of the velocities are computed by multiplying the recorded velocity and flow with the square root of the ratio of the standard micturition pressure to the measured pressure.

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*) As standard pressure we have chosen 64 cm of water, *i.e.*, the square number nearest 67, being the average value of the mean micturition pressure in our physiological patients.

A study of the physical laws applying to turbulent flow (Bretting 1940) shows, that the standard value of the urinary velocity must vary with the relative dimensions of the tube or, put in another way, with its steometrical form. The standard velocity of a smoothly calibered tube with no constrictions must be high and that of a tube with strictures low. The degree of deceleration provoked by a constriction will partly depend upon the ratio of the sectional area in the constricted part to the unconstricted part of the tube, partly upon the form of the constriction. A constriction which peripherally suddenly expands into a normal lumen will cause pronounced deceleration while a constriction with a protracted, conical course into a normal lumen will give only slight deceleration. The form of the passage from the "container" to the tube, in our case the shape of the bladder neck, as well as any bend in the tube, should only be of minor importance.

The relation between the standard values of velocity and flow will always be given by the formula: $Q = v \cdot F$, where F represents the cross sectional area in the urethral orifice. From this again it appears that the standard values of the flow are apt to vary with both the steometrical form and the absolute dimensions of the tube.

Taken as a whole it may thus be presumed that the standard value of velocity is applicable as an estimate for the presence of possible strictures of hydrodynamic significance, while the standard value of the flow is suitable as an expression of the absolute urethral conductibility.

It is, however, emphasised that what is written about the standard value of the velocity presupposes that possible strictures do not imply the external urethral orifice, and that the ratio between the length and the mean urethral diameter is tolerably constant from one patient to another. Apart from this, the remarks with regard to the standard value of the flow are applicable unconditionally.

PREVIOUS EXAMINATIONS

Until now about 40 measurements of the intravesical pressure during micturition have been reported. One author (Adler 1929) has performed the measurements through a cannula suprapubically inserted into the bladder but only gives scarce and insufficient information about the results. All other measurements seem to have been done by means of urethral catheters. Such catheters impede the urethral lumen and may, if the passage is already defective, cause micturition to be difficult or even impossible (von Garrelts 1958). Consequently nearly all previous measurements have been effected in normal males, and only in three instances, children or patients with stenosing disorders of the urethra or bladder neck, were subjects to examination.

With regard to the physiological intravesical pressure, Schwartz et al. (1922) and von Garrelts (1957) find that the pressure increases immediately prior to the onset of micturition, quickly reaches its maximum and then decreases evenly and slowly during the rest of micturition. Towards the end of micturition a brief, potential increase in pressure — after contraction (Denny Brown et al. 1933) — was often but not always, noticed. As regards the pressure's absolute maximum, Schwartz et al. (1922) find in seven males a maximal micturition pressure of 44—86 cm of water, in 11 males with urethral disorders of dubious character a pressure from 0 to "more than 90 cm of water", and in a 12 year old boy, suffering from enuresis, the pressure was "more than 90 cm of water". In an unspecified number of normal males Baumann (1955) records the mean pressure during micturition to be 105 cm of water and in a patient with prostatic hyperplasia an average pressure of 81 cm of water. Von Garrelts (1958) finds in 10 healthy young males a pressure of 60—102 and in a male with urethral stricture a pressure of 197 cm of water. On repeated recordings in the same individual with start of voiding at different bladder fillings the pressure was unaltered, from which he concludes, that the micturition pressure in each individual is quite constant.

The velocity of the urinary stream has been studied by Schwartz et al. (1922) by measuring the urinary "cast-distance". In seven normal males a velocity of 168—276 cm/s is recorded, and in 11 males with different urethral disorders a velocity of 132—378 cm/s. Numerous recordings in the same individual showed a considerable instability of the velocity and the deviations occurred independedly of the bladder filling prior to the micturition.

The most extensive and technically best performed examinations of the variations in the rate of flow during micturition seem to have been made by von Garrelts (1956,—57,—58). For this purpose he has constructed a flowmeter, which directly records the rate of flow as a function of the time. According to the description the apparatus is quite employable but rather expensive. He proves that the velocity in normal adults reaches a maximum of 10—35 cm^3/s in an even curve and drops markedly towards the end of voiding. In patients suffering from stricture of pars inferior urethrae he finds a more plateau-like curve and as a whole lower rate of flow, while the latter in patients with prostatic hyperplasia after a steep, initial rise decreases potentially during the whole micturition. Several measurements in the same normal individual show that the flow varies with the bladder filling before voiding. With less filled bladder the two magnitudes are directly proportional, but if the filling exceeds 250—300 cm^3 the flow approaches a maximal value asymptotically.

Studies with concurrent measurements of several hydrodynamic parameters are confined to 10 simultaneous determinations of flow and velocity in younger men (von Garrelts 1957). According to these the voiding starts during the initial rise in intravesical pressure and the after-contraction starts shortly before the end of micturition.

METHOD

It has been our intention to establish a simultaneous and continuous determination of the three hydrodynamic parameters: pressure, velocity and flow throughout micturition.

The pressure has been measured through a suprapubically inserted polyethylene-catheter (Sandøe, Bryndorf & Gertz, 1959), and has been recorded with a capacity manometer (Hansen 1949) and a selfwriting electrocardiograph ("Elema" Mingograph). The manometer's zero was brought to level with the lower edge of the symphysis.

The velocity has been determined by the course and length of the urinary stream, variations in the latter being registered by means of a film (8 mm film, 8 pictures/s). Determination of the velocity was made from every fourth picture, *i. e.*, every half second according to the following principles. If a horizontal jet was recorded — as was intended — the horizontal cast distance (x) and the vertical distance (y) from the urethra to the base were measured, then the velocity (v) was determined, from the formula: $v = x\sqrt{\frac{g}{2y}}$ (Schwartz & Brenner, 1922), where g represents the acceleration due to gravity, which is 981 cm/s². If the stream deviated from the horizontal plane we furthermore measured the angle (α) between the stream and the horizontal plane and then determined the velocity, using the formula: $v = x \cos \alpha \sqrt{\frac{g}{2(y - x \operatorname{tg} \alpha)}}$ or $v = x \cos \alpha \sqrt{\frac{g}{2(y + x \operatorname{tg} \alpha)}}$ depending on whether the stream at the start was directed above or below the horizontal plane. Film and pressure recording was timed by means of signals from the electromotor of the camera through one of the free channels into the electrocardiograph when the film was run.

Variations in the Rate of flow were more indirectly determined. At first the average flow (Q_{av}) and the average velocity (v_{av}) throughout micturition were computed. Q_{av} was derived from the ratio: $\frac{\text{volume of voided urine}}{\text{duration of micturition}}$ and v_{av} was worked out by the average of all determined velocities. Then the average sectional area (F) in the jet was determined by means of the formula $F = \frac{Q_{av}}{v_{av}}$. Finally the flow was established every half second during micturition by multiplying the simultaneous velocity value with F ($Q_t = F \cdot v_t$, when Q_t and v_t represent flow and velocity at the time t).

Most patients voided while standing, whereas smaller boys were placed in a lying or half-sitting position on a gynecologic couch and voided over the free edge. In order to provoke micturition the bladder was slowly filled through the catheter with physiological saline from a level container. In infants the filling was continued until the child voided, in adults the infusion was stopped when the patient was impelled to void.

MATERIAL

Nineteen males (aged from two months to 72 years) were examined. All were referred with suspected difficult vesical discharge. In most cases this was later either contradicted or verified through further examinations such as cystoscopy, micturition cystograms or operation. By means of all available information — except the result of the cystometry — we have tried to revise the diagnosis under which the patients were referred, and classify the patients in accordance with their urethral and vesical function. This classification resulted in four categories: 1) patients with normal conditions in both bladder and urethra; 2) patients with normal urethra but with signs of abnormally functioning bladder; 3) those with evidence of slightly stenosed urethra, and 4) those with severe urethral strictures. With the nomenclature *urethral stricture* we from now on in this paper understand every process in the bladder neck and the urethra impeding the discharge of urine.

The normal group comprises eight males from three to 28 years of age. The second comprises three children at the age of two, three and six years with considerable residual urine from 150—600 cm³. All three were later subjected to operation, during which rather identical conditions were revealed: a normal urethra through which fairly thick catheters could be inserted either way, a large thin-walled bladder, gaping ureteral orifices and hydro-ureters. The third category — patients presenting symptoms of moderate urethral stricture — consists of three males. A young man with 60 ml residual urine, haematuria and slightly trabeculized bladder. A 49 year old man with a history of only one attack of acute urinary retention, slightly enlarged prostate but no residual urine. And finally a 53 year old mand with chronic cystitis and 50 ml residual urine.

The fourth group comprises five patients in whom operation revealed severe stenotic processes in the bladder neck or the urethra. In two children — three months and one year of age — valves were found in the urethral pars membranaceae together with hypertrophic bladder wall, insufficient right-sided ureteral orifices and large

right-sided hydro-ureters. Three males — aged 57 to 72 years — presented hyperplasia of the prostate, hyperplasia of the prostate combined with sclerotic sphincter and a sclerotic sphincter alone.

Examinations, course and complications.

All examinations ran smoothly and in the majority it was fairly easy to insert the catheter percutaneously. By slow infusion of fluid, all patients were brought to void at least once, and usually two or three times during the examination, which lasted about two hours.

The voiding usually started at a high bladder filling, in most adults when 400—600 ml had been infused. Registration of pressure and determination of average rate of flow was carried out smoothly, while the film recording of velocity and the changes in the rate of flow turned out to be a rather laborious task. It was particularly difficult to make the photographic recording legible enough for the following measurement. In two instances it proved impossible, so that the determination was made by an approximate estimate of the observable cast-distance. Furthermore, before the technique of concurrent determination of velocity and flow had been worked out, only the voiding pressure and velocity were determined in two boys, and in one only the voiding pressure.

By repeated measurements we found in each single case a dispersion of the average values of the 3 hydrodynamic parameters from one micturition to another of about 10 per cent, and in all cases but one this dispersion did not exceed 25 per cent (Table 1). The latter was a patient with cystitis and dysuria, and it is conceivable that the increasing urethral resistance observed during micturition was due to pain spasms.

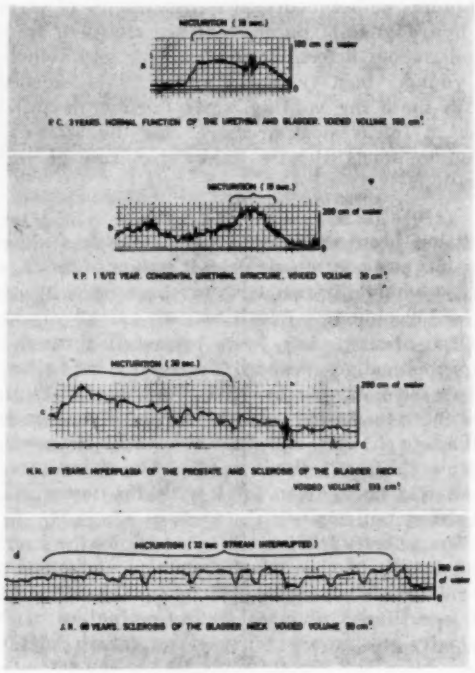

Fig. 1 a—d.
Intravesical Pressure Curve During Micturition. The Pressure is Measured with a Capacity Manometer and Continuously Recorded with a Self-writing Electrocardiograph.

RESULTS

Group 1. Normal cases.

Fig. 1a, 2a and 2b demonstrate curves from the normal cases. The intravesical pressure increases markedly before start of micturition, rapidly reaches maximum and then either maintains this level or decreases slowly towards the end of micturition, where it describes a steep drop. Furthermore we found in about half the patients a short but potential increase of pressure starting immediately prior to the end. In one case the after-contraction was seen as an inconstant phenomenon, only occurring during the first micturition but not in the second. The velocity started to rise a little later than the pressure, but otherwise the two parameters presented rather identical deviations. However, the after-contraction did not give rise to further micturition or acceleration. The average value of pressure during micturition varied in the normal patients from 50 to 80 cm of water with an average for the whole group of 67 cm of water. The velocity varied from 170—299 cm/s and the flow from 5—25 cm^3/s. The lower values were found in the smaller chidren and the highest in the adults, while the minor deviations seen in the pressure and velocity occurred rather unsystematically independent of age.

Group 2. Normal urethra, pathological bladder function.

The three children in this group were characterised by an unsteady voiding pressure and a varying flow and velocity throughout the micturition, while the mean values of all three parameters were almost the same as those in the normal patients.

Group 3. Signs of slight urethral strictures.

In these patients a rather high and somewhat changeable voiding pressure was seen together with a considerably lower velocity. The rate of flow was not or only slightly decreased due to an extraordinarily extended diameter in the urinary jet.

Group 4. Severe urethral strictures.

The five patients with pronounced urethral strictures showed fairly heterogeneous hydrodynamic conditions. Four patients had a very

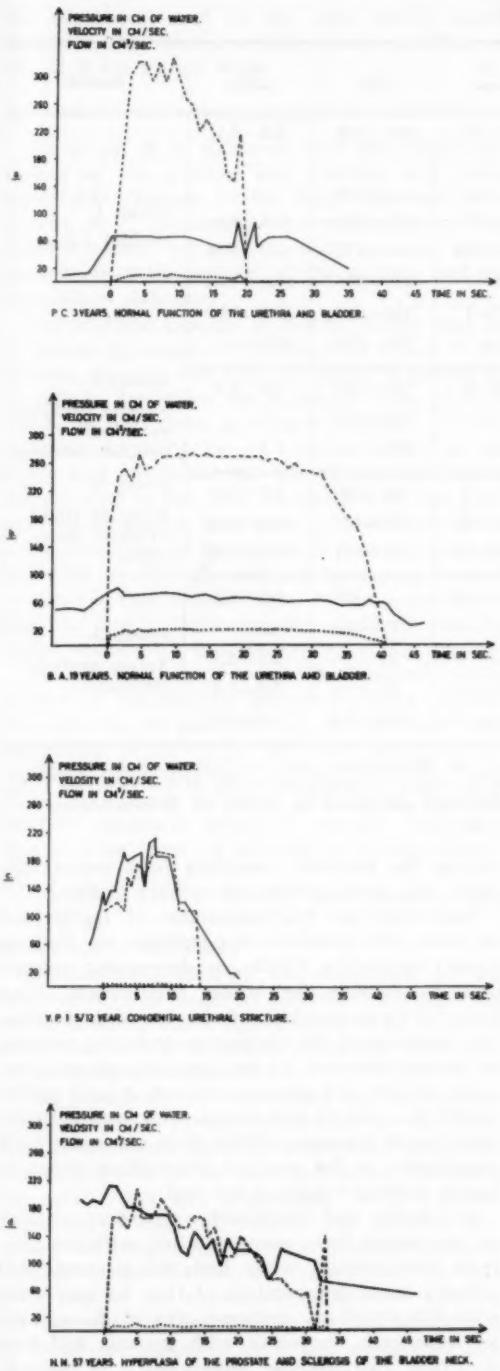

Fig. 2 a—d.

Graphs Demonstrating the Simultaneous Variations in the Intravesical Pressure, Urinary Velocity and Rate of flow. The Abscissas give the Time in Seconds, the Ordinates the Intravesical Pressure, the Urinary Velocity and Flow in cm of Water, cm/s and cm³/s, respectively.

high voiding pressure of 140—175 cm of water. Two of these, both children with urethral valves, presented a smoothly increasing pressure during the first half of the voiding followed by a slow decrease (Fig. 1 b) while the two older patients with prostatic hyperplasia presented a potential initial increase in pressure of more than 200 cm of water before the voiding really started, followed by a continuous decrease in pressure during the whole micturition (Fig. 1 c). The last patient in the stenotic group, an elder man with sclerotic sphincter, demonstrated a rather low pressure (65—85 cm of water) with a markedly unstable level, apparently due to insufficiency of the detrusor (Fig. 1 d). Following the observed variations in micturition pressure the two children with congenital valves presented a rather inert increase of velocity and flow during the first part of voiding and decrease during the last. The two patients with prostatic hyperplasia correspondingly showed an initiate steep rise in velocity followed by a continuous decrease throughout micturition (Fig. 2 d) and the patient with sclerotic sphincter presented an exceptionally short and interrupted urinary stream.

The average velocity varied throughout micturition from 40—160 cm/s, the average flow from 1.2—6.5 cm³/s. in the three adults and in the two children from 0.4—1.2 cm³/s. The sectional area of the streams was in all five cases very small, varying in the adults from 0.03—0.04 cm² against 0.07—0.11 in our normal adult patients.

Standard values of the velocity and flow.

In accordance with our preliminary theoretical considerations we have tried to determine the standard values of the velocity and flow as seen in Table 1. These values give a somewhat more distinct line of demarcation between the normal and the truly pathological cases, than that given by measuring directly. All normal patients have, regardless of age, a standard velocity value of 150 cm or more and all patients with heavy strictures have a standard velocity of less than 105 cm. Corresponding with this a standard rate of flow is found of more than 13 cm³/s in all physiological cases and only the patients with heavy strictures present a flow of less than five cm³/s. The conversion of the recorded values of velocity and flow to standard values gives a factor close to one in normal cases, while the factor in patients with urethral strictures often decreases to 0.7—0.6, due to the high intravesical pressure during micturition.

DISCUSSION

Owing to the development of the percutaneous method of catheterisation our material, contrary to earlier publications, comprises a rather large number of simultaneous determinations of micturition pressure, urinary velocity and flow, per-

Table 1.

Patient	Age	P cm of water	v cm/sec.	Q cm³/sec.	F mm²	v_{64} cm/sec.	Q_{64} cm³/sec.	Diagnosis
P. C.	3	61—65	243—259	5.2—8.1	2—3	247—259	5.3—8.1	
J. A.	4	50—60	—	—	—	—	—	
G. H.	7	69—80	— —170	—	—	— —150	—	
E. L.	7	57—68	195—220	9.7—10.0	5—5	206—215	10.6—9.8	Group 1
H. L.	8	65—66	— —180	14.7—17.8	— —10	— —177	14.4—17.4	Normal cases
B. I.	18	67—86	252—238	20.4—15.6	8—7	246—206	20.0—13.4	
B. A.	22	73—76	260—299	19.2—20.6	7—7	244—272	18.0—18.9	
S. M.	28	55—58	227—187	25.0— —	11— —	245—195	28.1— —	
A. A.	2	34—39	151—184	4.5—4.0	3—2	207—236	6.2—5.1	Group 2
M. P.	3	85—90	239—262	—	—	207—222	—	Normal urethra, pathological bladder function
J. C.	6	60— —	230— —	8.4— —	4— —	237— —	8.7— —	
J. S.	36	91—103	152—174	10.3—11.4	7—7	128—138	8.7—9.0	Group 3
J. P.	49	69—90	136—175	13.3—17.7	10—10	131—147	12.8—14.8	Signs of slight urethral strictures.
O. P.	53	76—136	146—162	19.5—20.2	13—12	134—111	17.9—13.9	
J. F.	3/12	165—175	— —80	0.4—0.7	— —0.9	— —48	0.2—0.4	
V. P.	1	160— —	140— —	2.0— —	1— —	87— —	1.2— —	Group 4
H. N.	57	140—160	120— —	4.7—4.5	4— —	81— —	3.1—2.9	Severe urethral strictures.
J. K.	68	65—85	40— —	1.2—1.5	3— —	39— —	1.2—1.3	
F. T.	72	148—150	153—160	6.0—6.5	4— 4	100—104	4.0—4.3	

Average Values Throughout Micturition: (p) = Pressure of Micturition, (v) = Urinary Velocity, (Q): Rate of Flow, (F) = Cross Sectional Area in the Urinary jet, $(v_{6\,4})$ = Standard Value of Velocity, $(Q_{6\,4})$ = Standard Value of Flow. Results from Repeated Measurements are Given as limits of Determination.

formed in both physiological and pathological cases, and in infants as well as adults.

In each individual case the three hydrodynamic parameters seem to remain constant from one micturition to another, irrespective of age and character of disease. Taking into consideration that all our examinations are performed at relatively high bladder filling, this corresponds with the observations made by von Garrelts (1957) concerning pressure and flow, but not with those made by Schwartz & Brenner (1922) concerning the velocity.

However, considerable hydrodynamic deviations appear from one patient to another. In cases with both normal urethra and bladder the pressure during micturition was quite steady and the urinary velocity and flow quite constantly high, whereas the pressure in patients with a weak and insufficient detrusor was more unstable and the urinary velocity correspondingly changeable. In cases with faulty urethral passage the bladder seemingly tried to force the urine through by intensifying the voiding pressure. Nevertheless the pressure compensation was not complete, for which reason the velocity and flow in most stenosed cases were lower than normal. In the case with sclerotic sphincter the detrusor seemed inefficient and incapable of raising the pressure, resulting in a remarkably short, thin and interrupted urinary stream.

Regarding the hydrodynamics of micturition in cases with prostatic hyperplasia, we find, as von Garrelts (1958), a decreasing urinary flow throughout the whole micturition. Von Garrelts assumed this to be due to an increasing resistance in the bladder neck during voiding, but as demonstrated by our simultaneous measurements of flow and pressure it must, at least partly, be due to a steady and pronounced decline in the intravesical pressure, which from extremely high magnitudes at the start of micturition drops to nearly normal values at the end.

In infancy and childhood we find as a whole an increasing flow, corresponding with the physical development, while both the pressure and velocity seem independent of this. In agreement with this the cross sectional area of the urinary jet apparently increases with growth, but here the fact furthermore supervenes, as already known from the clinic, that all patients with urethral strictures present very thin streams. Due to this a urethral stricture should reduce the urinary flow relatively more than the velocity. The most likely explanation of this phenomenen is, that the gradient of pressure across the stricture is so steep that no pressure is left to dilate

the peripheral part of the tube, which partly collapses, thus giving a thin urinary jet (Morales & Romanus 1953).

CONCLUSION

All in all it is apparent that functional disorders of the urethra and bladder will cause recordable changes in the hydrodynamic parameters, or — conversely — measurements of the hydrodynamic parameters will provide information about the passage of the urethra and the function of detrusor.

The urethral passage or conductibility may be estimated by means of the standard values of the urinary velocity and flow. In adults it should be enough to determine the standard value of flow alone, which gives a relative estimate of the urethral conductibility in absolute units. In infancy and childhood the conditions seem more complicated as the flow increases with the physical development, thus making it difficult from a determination of the standard flow value alone to decide whether the urethral passage is normal or not. For that reason it may pay in a paediatric clientele also to determine the standard velocity which apparently is independent of the growth.

The significance of using the standard values instead of the directly recorded values of flow and velocity as estimates of the urethral conductability is shown by the large dispersion in the micturition pressure in this material, from 33 cm to 175 cm of water. This means that the directly recorded values of velocity and flow may give an error of estimate of approximately $\frac{100 \cdot \sqrt{170} - \sqrt{34}}{\sqrt{34}} = 124$ per cent, presupposing turbulence of the flow.

Information regarding the efficiency of the detrusor may be deduced directly from the form of the pressure curve and possibly also from the magnitude of the pressure compared with that of the urethral resistance.

The possibility of examining the hydrodynamic parameters of the micturition in the practical clinic seems feasible. Suprapubic catherisation appears to be an easy procedure, involving but small risks and practicable in all patients regardless of age and vesical and urethral disorders. To this comes the fact that the pressure changes in the bladder are rather slow, so that the sensitive, but expensive manometer used in our investigation presumably can be substituted for practical purposes by a less expensive, mechanical manometer. Determinations of the average velocity and flow can always be effected and demand no special apparatus. Velocity is computable by means of the observable "cast distance" and the flow by the ratio of the volume of voided urine to the duration of voiding. On the other hand a continuous recording is difficult and according to our experience hardly necessary, because the urethral resistance seems to be fairly constant during the major part of the voiding. In other words a cannula, a small plastic catheter with stylet, a manometer such as that used in lumbar punctures, a yardstick and a measuring glass are the simple utensils required for the examination, which in most cases can be made in less than two hours.

SUMMARY

The hydrodynamic parameters of the micturition, *i. e.*, the intravesical pressure, the urinary velocity and the rate of flow during micturition, vary in accordance with the function of the bladder and urethra. This implies that in most cases a simultaneous determination of all three, or perhaps only the pressure with either the urinary velocity or rate of flow, may give a complete picture of both the urethral conductibility and the bladder function.

The determination can be performed with a simple, inexpensive apparatus, in a fairly short time, and should therefore be of practical diagnostic importance in patients with urethral and vesical disorders.

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ABO BLOOD GROUPS AND STOMAL ULCER

By BJØRN BLEGVAD

The possible relationship between blood groups and disease has been the subject of study for many years. Particularly after the publication of Aird, Bentall & Roberts' investigations (1953) into the blood group distribution in gastric carcinoma, numerous papers have seen the light of day. In this type of study, it is often difficult to procure sufficiently large groups of patients.

By adding up the results of several investigations, it is possible to accumulate so large a number of patients that the results can form the basis of reliable statistical analysis.

It seems justified, therefore, to publish such analyses, so that others, by adding the figures to their own, might be able to achieve greater statistical accuracy.

PREVIOUS INVESTIGATIONS

In 1957 Bentall published an investigation of the distribution of blood groups in 159 patients with stomal ulcer, showing a significant increase of group O in these patients compared with the frequency of group O among duodenal ulcer patients.

His series was collected from 3 British centres, comprising 79 previously published cases from Glasgow (Peebles Brown et al. 1956). The results were presented as follows (Table 1).

Table 1.

Distribution of ABO Blood Groups in Patients With Stomal Ulcer Compared With the Distribution in Duodenal Ulcer Patients. Great Britain.

Centre	Stomal ulcer		Duodenal ulcer		Increased relative incidence in group O	χ^2
	O	A+B + AB	O	A+B + AB		
London	35	18	506	394	1.54	1.94
Newcastle ...	16	11	281	186	0.96	0.01
Glasgow	55	24	947	695	1.68	4.34
	159					6.29

Mean increase in incidence 1.46.

	D. of F.	χ^2
Difference from unity	1	4.88
Heterogeneity	2	1.41

From Department A, Bispebjerg Hospital, Copenhagen. (Head: *K. H. Køster)* and Department I, Kommunehospitalet, Copenhagen. (Head: Prof. *O. Mikkelsen* †).

MATERIAL

The present author studied the distribution of blood groups in 164 stomal ulcer patients from two surgical departments in Copenhagen: Department A, Bispebjerg Hospital and Department I, Kommunehospitalet. The series is derived from the years 1945—58 inclusive. All doubtful cases were excluded. In 145 cases the diagnosis was confirmed by operation, in 2 cases by gastroscopy, in 2 cases by autopsy, and in the remaining 15 cases by X-ray evidence of a definite ulcer crater.

The results were compared with previous investigations into the blood groups in duodenal ulcer patients from the same two departments, from Bispebjerg Hospital covering the period 1947—54 (Køster et al.), and from Kommunehospitalet 1949—55 (Jordal). These investigations had shown a significant excess of group O in comparison with the control group of healthy people in Copenhagen (Bryde Andersen).

Both hospitals receive their patients from the same groups of population and from the same geographical area. There is some difference in the blood group distribution between the stomal ulcer cases from the two departments, but this difference is not significant ($\chi^2 = 3.44$, d. of f. = 1). There was no significant difference between the two groups of patients with duodenal ulcer.

The blood group determinations were carried out by the same institutions as in the previous investigations (by Statens Seruminstitut up to 1950, and after 1950 by the Copenhagen Municipal Blood Banks).

Table 2.

Distribution of ABO Blood Groups in Patients With Stomal Ulcer Compared With the Distribution in Duodenal Ulcer Patients. Copenhagen.

Hospital	Stomal ulcer		Duodenal ulcer		Increased relative incidence in group O	95 per cent fiducial limits
	O	A+B + AB	O	A+B + AB		
Bispebjerg ..	45	41	342	338		
Kommunehosp.	51	27	588	635		
	96	68	930	973		
	164		1903		1.43	1.06-1.93

RESULTS

The results of the investigation are shown in Table 2. Like the British authors, the present

author found a significantly higher proportion of group O in patients with stomal ulcer (O = 58.5 %) than in patients with duodenal ulcer (O = 48.9 %) ($\chi^2 = 5.26$, $p < 5$ %, d. of f. = 1). The increased incidence in group O in the Danish patients (1.43) is almost identical with the mean increase in incidence in group O found among the British patients (1.46). In a small series like ours, the 95 per cent fiducial limits to this quotient are fairly wide: 1.06—1.93. Combining the British and Danish results gives a mean coefficient of 1.45 and a narrowing of the limits to 1.19 and 1.77. Our series thus seems to support Peebles Brown's and Bentall's assumption of a relationship between the blood groups and the severity of the ulcur diathesis.

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ABO BLOOD GROUPS AND GASTRIC ACIDITY

By BJØRN BLEGVAD

PREVIOUS INVESTIGATIONS

Køster et al. (1955) studied the blood group distribution in patients with duodenal ulcer, gastric ulcer and gastric carcinoma as well as in normal subjects, and at the same time they made an estimate of the gastric acidity in these conditions. They combined the results of the two studies in the following diagram (Fig. 1). The estimate of the acid production had been based partly on these authors' own observations, partly on previous reports. The investigations were carried out at the Bispebjerg Hospital, Department A.

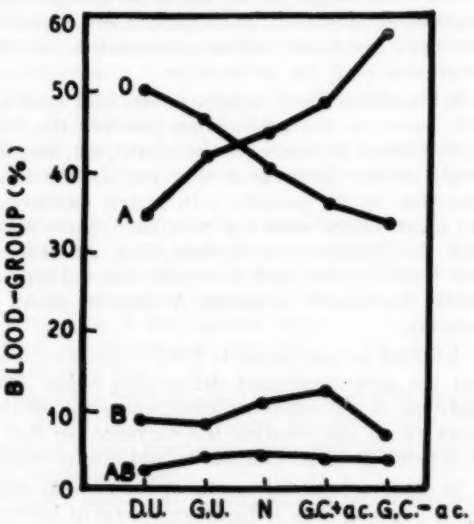

Fig. 1.

Frequency of blood groups O, A, B, and AB in five different conditions of the stomach and duodenum arranged in order according to the output of gastric acid.

D.U. = duodenal ulcer. G.U. = gastric ulcer. N = normals.
G.C. + ac. = gastric carcinoma with achlorhydria.
G.C. — ac. = gastric carcinoma without achlorhydria.

From Department A, Bispebjerg Hospital, Copenhagen. Head: *K. H. Køster.*

Proceeding from left to right on the diagram, *i.e.*, from the conditions associated with the highest to those associated with the lowest acid secretion, we find an increasing frequency of group A and simultaneously a decreasing frequency of group O.

Accordingly, a direct relationship might be assumed to exist between ABO blood group and gastric acid output.

This is supported by the findings of Buckwalter et al. (1956). Among a group of 529 peptic ulcer patients who had histamine tests, they found the frequency of group O to be significantly greater among those who reacted with an increase of free hydrochloric acid than among those in whom no increase of acid occurred.

Peebles Brown (1956) studied the output of hydrochloric acid in 230 duodenal ulcer patients and 46 gastric ulcer patients by an "augmented" histamine test by the method of Kay and analysed the results according to blood group. In this series of 276 patients *no* correlation between acid output and blod group was found. (In both groups there was a slightly higher acid output in group O than in group A patients, but the differences were not significant).

In cancer of the stomach, Buckwalter et al. (1957) found no significant association between ABO blood groups and acid output. They do not supply further data.

The theory of such an association has also been opposed by Billington (1956) and Jennings & Balme (1956 and 1957) in their publi-

cations on blood groups in gastric ulcer and gastric carcinoma. Their findings indicated a correlation between blood group and the *site* of the lesion (antrum, body, cardia) rather than between blood group and the *nature* of the lesion (cancer, ulcer) as previously assumed. However, Haddock & McConnell's (1956) findings did not accord with this result.

METHOD AND MATERIAL

During the past two years patients admitted to Department A, Bispebjerg Hospital, suffering from gastro-duodenal disease have been submitted to "augmented" histamine tests for determination of their hydrochloric acid production*).

The procedure has been described by Køster & Thorsøe. Histamine + antihistamine is administered by continous intravenous infusion in doses so large as to secure a maximum stimulation of the gastric acid production. The method does not differ materially from Kay's in which, however, histamine is administered by the subcutaneous route.

It seemed of interest to investigate whether these patients showed any difference in acid production according to blood group (O, A, B, and AB).

The series comprises 270 patients: 89 with duodenal ulcer, 102 with gastric ulcer, 18 with gastritis, and 61 with gastric carcinoma. In all cases, the diagnosis was confirmed by operation. Blood grouping was carried out at the Blood Bank, Bispebjerg Hospital.

The hourly output of mEq H^+ was as shown in Table 1.

Table 1.

Acid Output in Patients With Gastro-duodenal Lesions According to ABO Blood Groups

Duodenal ulcer.

Blood group	n	M	diff.	t.
O	41	30.46 mEq H^+/h		
A	37	27.61 »	2.85	0.86
B	6	31.40 »	—0.94	—0.15
AB	5	23.89 »	6.57	0.95
		s^2 = 212.23		

Gastric ulcer.

Blood group	n	M	diff.	t.
O	44	12.96 mEq H^+/h		
A	39	16.05 »	—3.09	—1.27
B	12	12.88 »	0.08	0.02
AB	7	12.51 »	0.45	0.10
		s^2 = 122.29		

Gastritis.

Blood group	n	M	diff.	t.
O	7	13.42 mEq H^+/h		
A	11	14.79 »	—1.37	—0.34
		s^2 = 67.60		

Gastric carcinoma.

Blood group	Patients, total	No. of patients with achlorhydria	Achlorhydria in per cent of patients	diff.	t.
O	23	11	47.8		
A	27	16	59.3	—11.5	—0.81
B	6	2	33.3		
AB	5	2	40.0		
	61	31	50.8		

n = number of patients.
M = mean of values representing acid output.
s^2 = estimate of variance.
diff. = difference from mean value in group O.
t = difference devided by standard error.
5 per cent limit for t is about 2.

*) The first 143 patients were tested by *H. Thorsøe.*

RESULTS

The findings indicate a normal log. distribution of the acid output in the different groups. Log. calculation, however, gives practically speaking the same result as ordinary calculation, so the latter was used for publication.

In duodenal ulcer, gastric ulcer, and gastritis differences in the acid output between the four named blood groups were not significant, and the trend of the differences was not in the same direction. In the patients with gastric carcinoma, the mean values were not calculated, since about half the patients had achlorhydria. Absence of free hydrochloric acid, however, was not significantly commoner in group A than in group O patients.

Addition of our series to Peebles Brown's did not give significant differences either. This addition is not included here, since the British workers do not mention the variance, so that it is not definitely permissible to add up the results.

In other words, the results of the present study do not in any way indicate relationship between ABO blood group and gastric acidity.

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ABO BLOOD GROUPS AND FRACTURE OF THE FEMORAL NECK

By HANS THORSØE

In a search for a suited control series in blood group studies Buckwalter et al., in 1957, found to their surprise that among patients with fracture of the neck of the femur, unlike those with other fractures, there was a significant excess of group A at the cost of group O individuals.

Their series comprised 981 patients, but they did not study which types of fractures the patients had sustained or the possible relationship between type of fracture and blood group.

MATERIAL

During the period 1949 to 1960 a total of 707 patients (182 males and 525 females) with fracture of the neck of the femur were admitted to the four surgical departments of the Bispebjerg Hospital, Copenhagen.

On the basis of the type of fracture, the patients were divided into three groups:

1) Patients with lateral fractures, comprising pertrochanteric and intertrochanteric fractures.
2) Patients with stable valgus fractures.
3) Patients with unstable varus fractures, subcapital as well as transcervical. This is the type which is identical with the classical fracture of the femoral neck.

This classification is very close to Anschütz & Potwich's.

Out of the 707 patients, 216 had lateral fractures, 114 stable impacted valgus fractures, and 377 classical varus fractures. The ABO blood group was studied in 590.

Table 1 shows the distribution of blood grouped and non-blood grouped patients within the three types of fracture. 117 or 16.5 per cent had not had blood group determination. The fact is that during the years 1949 and 1950 this was not done as a routine, not even in cases treated by operation. Half the patients with valgus fracture, moreover, were not blood grouped, because they were not treated by operation.

So, the results must be regarded with some reserve, especially in evaluating the distribution of ABO groups in patients with fracture of the femoral neck *versus* other categories of patients. In an assessment of the findings in classival *versus* lateral fractures, on the other hand, the error caused by the non-blood grouped patients may be practically speaking disregarded, since the proportion of patients with classical fractures and lateral fractures was the same among blood grouped and non-blood grouped cases (cf. Table 1). Thus, there can be no question of selection.

Table 1.

Distribution of Blood Grouped and Non-blood Grouped Patients Within the 3 Sub-types of Femoral Neck Fractures.

	Blood grouped	Not blood grouped	Total
Lateral fractures	192	24	216
Valgus fractures	64	50	114
Classical fractures ...	334	43	377
Total	590	117	707

Distribution of Blood Groups in Normal Subjects.

In 1955 the blood group distribution among 14,304 healthy persons in Copenhagen Bryde Andersen analyzed. All the determinations had been carried out at the Blood Bank of the Bispebjerg Hospital.

These persons represent a cross section of the adult Copenhagen population, between 15 and 70 years of age. All our patients were Copenhagen residents.

In 1927 the blood group distribution among elderly persons between 65 and 102 years of age was analyzed in a study comprising only 609 persons who were specially selected.

Table 2 gives the distribution of blood groups in the two control series. Between these two series there are no significant differences. We preferred the former as a basis of comparison, because it was larger and because it comprised

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Table 2.

Distribution of Blood Groups Among 2 Series of Normal Persons (1) Aged 15—70 Years and (2) Aged 65—102 Years.

Age	Number of subjects	O per cent	A per cent	B per cent	AB per cent
15— 70 ..	14,304	40.6	44.0	10.9	4.5
65—102 ..	609	44.0	42.0	9.2	4.8

non-selected healthy persons from the area where our patients lived.

RESULTS

The distribution of ABO blood groups among 590 patients with fracture of the femoral neck is shown in Table 3. There is no excess of group A persons.

Table 4 shows that group A is less common among patients with lateral fractures of the femoral neck than among the control series. However, the *u* value, indicating the divergence from normal, divided by the standard error, is not significant, although it approaches the 5 per cent limit.

Table 3.

Distribution of Blood Groups Among 590 Patients With Fracture of the Femoral Neck.

Blood group	No. of subjects	Per cent	S. D.	Per cent distribution in control group	Divergence from normal	u
A ..	262	44.4	± 2.0	44	+ 0.4	0.2
O ..	239	40.5		40.6	— 0.1	
B ..	67	11.3		10.9	+ 0.4	
AB .	22	3.8		4.5	— 0.7	

Table 4.

Distribution of Blood Groups Among 192 Patients With Lateral Fractures of the Femoral Neck.

Blood group	No. of subjects	Per cent	S. D.	Per cent distribution in control group	Divergence from normal	u
A ..	72	37.5	± 3.5	44	— 6.5	— 1.9
O ..	92	47.9		40.6	+ 7.3	
B ..	25	13.0		10.9	+ 2.1	
AB .	3	1.6		4.5	— 2.9	

Table 5.

Distribution of Blood Groups Among 334 Patients With Classical Fractures of the Femoral Neck.

Blood group	No. of subjects	Per cent	S. D.	Per cent distribution in control group	Divergence from normal	u
A ..	171	51.2	± 2.7	44	+ 7.2	2.6
O ..	109	32.6		40.6	— 8	
B ..	35	10.5		10.9	— 0.4	
AB .	19	5.7		4.5	+ 1.2	

Table 5 gives the distribution of ABO groups in patients with classical fractures of the femoral neck among whom there is a significant excess of group A patients. The *u* value is significant even in respect to the 1 per cent limit.

According to Barnet Woolf the incidence ratio between group A and group O is 1.47 in classical types of femoral neck fracture.

DISCUSSION

The literature does not give a definite impression of the genesis of femoral neck fracture.

Key & Convell (1936) as well as Apfelbach & Aries (1937) have advanced the theory that the underlying process is a disease of the femoral neck. In their opinion, a group of people would more easily than others sustain fracture of the femoral neck, *e.g.* merely by stepping rather firmly from the curb, the fall being secondary.

Especially patients with the classical varus fracture often have a history of only very slight injury. In some cases the fracture has even resulted from vertical stress. According to most authors, vertical stress prediposes to lateral fractures, unless the subject has a predisposition to varus fractures.

In our study, there was a significant excess of blood group A among patients with the classical fracture of the femoral neck. This might indicate that some of the patients with classical fractures had a prediposition to such fracture.

For the purpose of studying this aspect, we had planned to ascertain whether any relationship existed between the severity of the trauma and the type of fracture. This plan had to be abandoned, however, because of insufficient data in the case records.

The only previous paper on this subject Buckwalter et al. 1957), does not mention the types of fracture of the femoral neck. Consequently, their results are not directly comparable with ours. That Buckwalter et al. found a significant excess of group A among patients with fracture of the femoral neck and we did not may be due to a larger number of patients with classical fractures in their series.

SUMMARY

In a series of 707 patients with fracture of the femoral neck we did not find any major excess of blood group A. On the other hand, there is a distinctly significant difference between the distribution of groups A and O among the various sub-types of fractures, group A predominating among patients with the classical type of fracture.

This distribution might indicate that the aetiology of fracture of the femoral neck is not exclusively mechanical.

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The statistical calculations given in the above three papers on blood groups and disease were carried out by *Mogens Nyholm,* Actuary.

GENERALIZED GLYCOGENOSIS

By JØRGEN KRINGELBACH

By *glycogenosis* is understood an abnormally high deposition of glycogen in one or more organs as a result of disturbed function in the normal intracellular carbohydrate metabolism enzyme system.

The best known and the most frequent of the glycogenoses is *van Creveld—von Gierke's disease.* This is a hepato-renal glycogenosis characterized by hepatomegaly, hypoglycaemia, ketonuria, abnormal glucose tolerance test of a diabetic type and absence of increase in the blood sugar level on stimulation with adrenaline (12).

Cori (3) showed, by means of her investigations concerning the structure of glycogen and the significance of the enzyme system for the metabolism of glycogen, that van Creveld—von Gierke's disease is due to reduced or absent glucose-6-phosphatase activity and thus is definitely differentiated from the cardio-muscular, or more correctly, generalized glycogenosis in which normal glucose-6-phosphatase activity is found.

The cause of generalized glycogenosis is still unknown. The glycogen is of normal structure. It is presumed that the condition is due to an enzymatic defect but this has not hitherto been demonstrated. Cori mentions as a possibility that the condition could be explained by a slight reduction in the quantity of phosphohexoisomerase or phosphofructokinase, and says that such a reduction would be difficult to find and evaluate. A slight reduction in the quantity of an enzyme such as this would result in reduction in the rate of the destruction of glycogen which, provided that normal glycogen formation was present, might lead to gradual accumulation of glycogen, particularly in the muscles and in the heart, exactly as is the case in generalized glycogenosis.

From The Paediatric Department, The Central Hospital, Nykøbing Falster (Head: *Jørgen Kringelbach).*

Generalized Glycogenosis (Synonyms: "Glycogen Storage Disease of the Heart", Pompe's syndrome, Cardiomegalia glycogenica diffusa etc.) is a rare disease of infancy. Hitherto, about fifty proved cases have been described and of these the great majority were diagnosed *post mortem.*

The condition is a recessive hereditary disease, which has been demonstrated in siblings in several instances (1, 4, 6, 8) and in some cases also there was consanguinity between the parents (4, 11).

SYMPTOMS

Symptoms may be present already at birth but, as a rule, they do not appear until the infant has attained the age of some weeks to some months.

The disease becomes apparent by failure to thrive, increasingly poor general condition with motor retardation, muscular hypotonia with weak or absent tendon reflexes. Many such infants have macroglossia. The cardiac symptoms consists of increasing dyspnoea and attacks of cyanosis frequently in connection with feeds. There is a great tendency to respiratory infection.

Objectively, such infants are found to be very pale with poor muscular tone. Occasionally, the bulk of the muscles provides a sharp contrast to this. In such cases, the musculature feels strikingly firm and nodular. Examination of the heart, as a rule, shows increased cardiac dullness and frequently tachycardia but only rarely murmurs. As a rule, the liver is not strikingly large.

Radiographic examination of the heart shows it to be enlarged and frequently enormous. The heart is globular and diffusely enlarged. The *electrocardiogram* shows signs of myocardial damage. From the limited number of electrocardiograms taken in such patients the majority show narrow QRS complexes with great amplitude, lowered ST segments in the leads from the extremities and several of the precordial leads

and pointed inverted T waves in the same leads and, finally, the conduction time is found to be short in numerous cases. Other patterns have also been observed, however. (7, 9, 10, 11, 13, 14).

Other laboratory investigations indicate, by and large, normal findings in all other respects and, in particular, the normal fasting blood sugar, normal glucose tolerance test, normal adrenaline tolerance test and the absence of acetone or diacetic acid in the urine should be stressed (5, 13).

Course of the disease:

The average age at death is four months with variations from a few days to nine to ten months. The usual cause of death is cardiac insufficiency or respiratory paresis associated with terminal bronchopneumonia and atelectasis.

Post Mortem Findings:

The heart is found to be globular and enlarged with uniform diffusely enlarged ventricles while the atriae are not enlarged. The cardiac muscle is pale red. Histologically, the myocardial fibers reveal a "lacework" appearance which are due to the fact that all the myocardial fibers are massively infiltrated with glycogen and appear as hollow cylinders surrounded by a very fine membrane of cytoplasm. In a similar manner, the striated muscles are filled with glycogen-containing vacuoles. Accumulation of glycogen is also found in the muscular tissue of the intestinal tract and the vessels, in nervous tissue and in practically all of the systems.

Problems in differential diagnosis:

In some cases, the cardiac lesion may entirely dominate the clinical picture while other cases have been described in which the disease has manifested itself as a purely muscular condition (16). In cases of hypertrophy of the heart the cardiomegaly of glycogenosis cannot be differentiated with certainty, solely by radiography and electrocardiography, from e.g. endocardial fibroelastosis or abnormal origin of the left coronary artery. Muscular biopsy can reveal whether glycogenosis is concerned.

The cases in which muscular affection predominates have been misdiagnosed as amyotonia congenita or muscular dystrophy and when macroglossia is pronounced, diagnoses such as cretinism and mongolism have been made (2, 6, 16). In these cases also, the diagnosis may be established by muscular biopsy. The tissue should be fixed in absolute alcohol to retain the glycogen content. This may be demonstrated by staining with carmine by Best's method.

CASE HISTORY

The patient was a boy aged 3½ months on admission to the Paediatric Department. The infant was the second child of healthy parents. The father was 39 and the mother 27 years of age. The parents were first cousins, the infant's maternal and paternal grandfathers being brothers. The patient's brother, aged two years, was healthy. There were no cases of cardiac disease nor sudden death among children in the family. The mother had been healthy during the entire pregnancy. The delivery occurred at the expected time and was uncomplicated. The birth weight was 3500 g (7 lb. 12 oz.) and length 52 cm. There was no asphyxia. The infant was breast-fed for three weeks and, thereafter, received mixed feeding until the age of two months. The infant thrived well until the age of six weeks (See Figure 6). From the age of approximately six weeks increasingly frequent thin greenish stools were observed and, simultaneously, the infant lost appetite, became whining and weak and lost some weight. At the age of two months the infant was admitted to the local hospital on account of dyspepsia. In this hospital, on acount of the appearance, the infant was first thought to be an imbecile and, later, cretinism was suspected. X-ray photographs, taken to show the centres of ossification, revealed a strikingly large heart. The infant was then transferred to The Paediatric Department in The Central Hospital.

Figure 1.
Macroglossia.

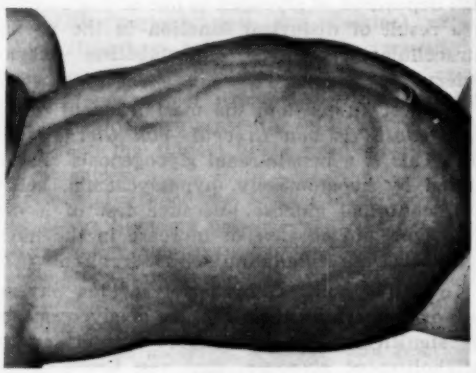
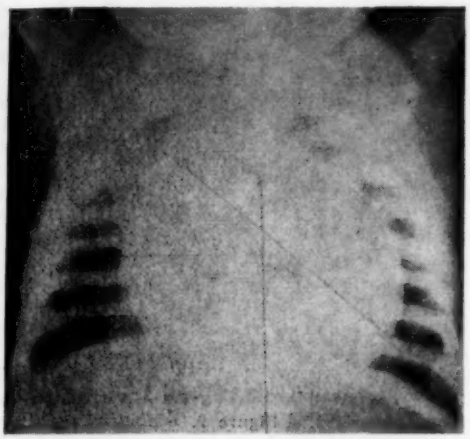
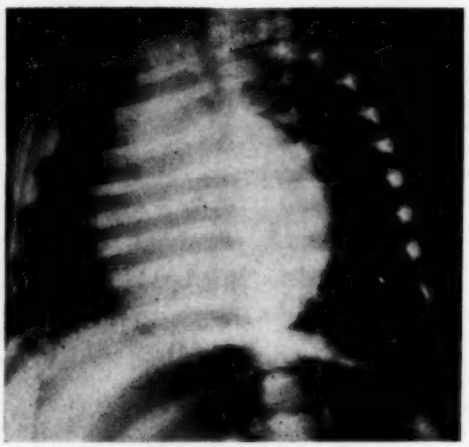


Figure 2.
Pseudohypertrophic abdominal musculature.





Figures 3 and 4.
Globular hypertrophy of the heart.

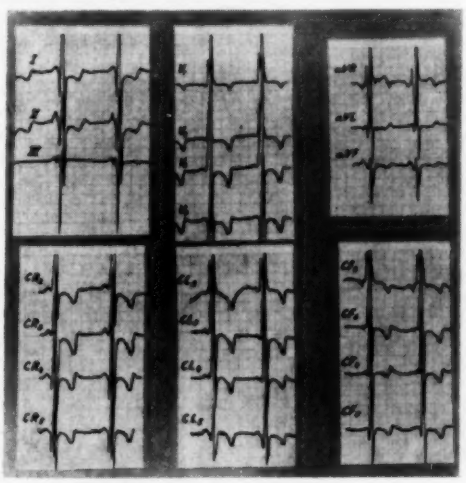


Figure 5.
Electrocardiogram showing depressed ST segments, inverted T waves, narrow QRS complexes with very great amplitude and P-R of 0.07 second.

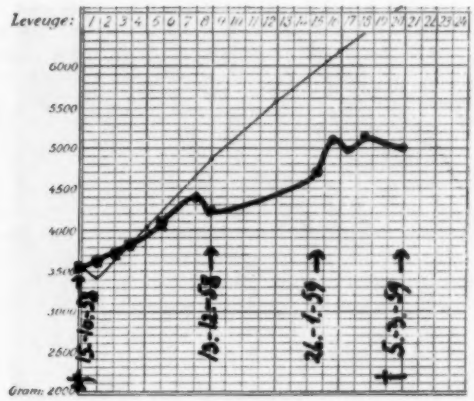


Figure 6.
Weight curve of the patient compared with a normal curve. The two dates in the middle indicate admission to the local and the Central Hospitals, respectively.

On admission, the infant showed a greyish pallor and was thin and markedly relaxed. Simultaneously, the musculature was hypotonic and pseudohypertrophic. The infant made only rare spontaneous movements and the tendon reflexes were practically absent. There was pronounced macroglossia and the tongue was constantly protruded between the lips (Figure 1). Examination of the heart revealed increased cardiac dullness. On stethoscopy the day of admission, the impression of gallop rhythm with a moderate systolic murmur was obtained. Later, the stethoscopic findings were normal on repeated occasions. The infant was catarrhal but could not cough adequately. The abdomen was somewhat meteoritic and enlarged and the recti abdomini could be distinctly seen with pronounced tendinous intersections (Figure 2). The liver margin was felt 2—3 cm below the costal margin but no impression of pathological enlargement of the liver was obtained. The spleen and kidneys could not be felt. A left inguinal hernia was present.

X-ray examination revealed a greatly enlarged globular heart (Figures 3 and 4). The electrocardiogram revealed depressed ST segments in Leads I and II and in several precordial leads, pointed inverted T waves, narrow QRS complexes with great amplitude and the P-R interval was 0.07 second (Figure 5). From these findings it was deduced that the infant suffered from cardiomegaly due to glycogenosis and the diagnosis was confirmed by biopsy (See later).

Weight curve: (Figure 6). Length 59 cm. Circumference of head 40 cm. Circumference of thorax 38 cm.

Hb. 100 — 76 — 83 %. Erythrocyte count: 4.33 → 3.15 million. Leucocyte count: 15,880 → 9,520. Differential count: moderate neutrophile leucocytosis. ESR (micro): 7—6 mm. Wassermann reaction negative. Moro's tuberculin test negative.

Urine: No albumin, blood, sugar, acetone, diacetic acid nor urobilin. Diastase < 150. Microscopy revealed no abnormality.

Creatinin 0.6 mg %. Cholesterol 119 mg %. Thymol 0.06. Transaminase (GO-T): 372. Calcium 9.2 mg %. Phosphorus 5.3 mg %. Alkaline phosphatases 15.9, acid phosphatases: 7.3. Chloride: 94 mEq. Sodium: 127 mEq.

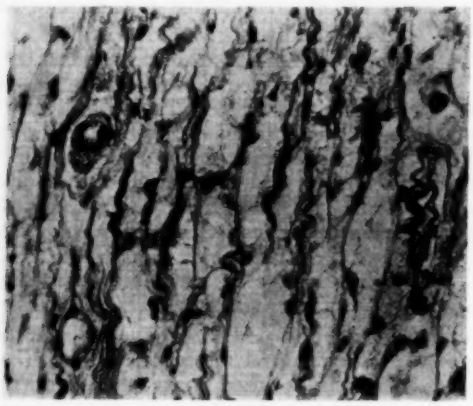


Figure 7.
Striated muscle with marked vacuolization (Magnification approximately × 600).

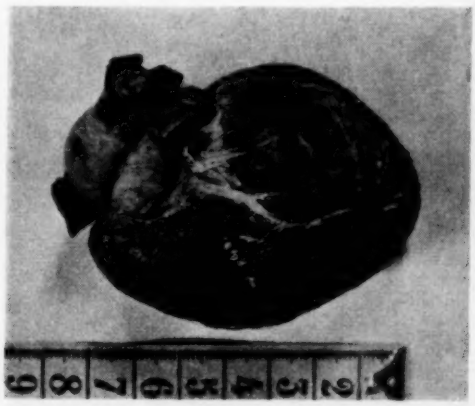


Figure 9.
The entire heart.

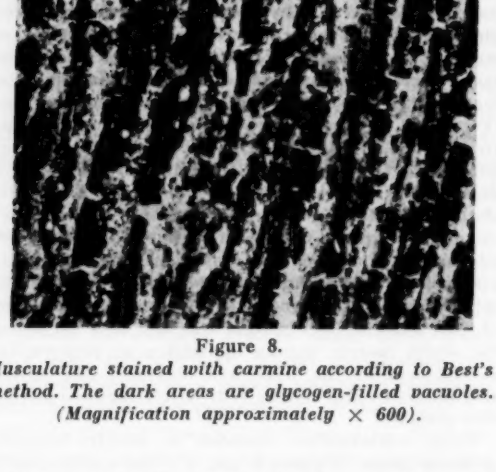


Figure 8.
Musculature stained with carmine according to Best's method. The dark areas are glycogen-filled vacuoles. (Magnification approximately × 600).

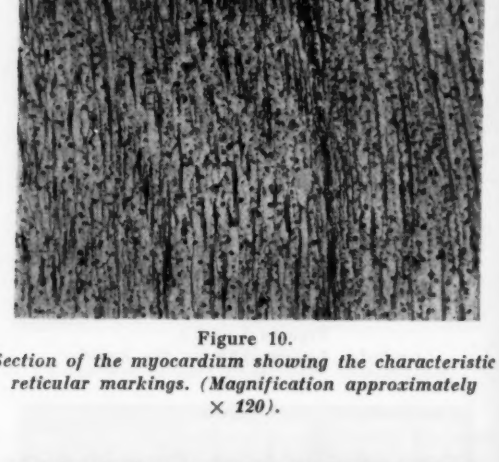


Figure 10.
Section of the myocardium showing the characteristic reticular markings. (Magnification approximately × 120).

Potassium: 5.5 mEq. Fasting blood sugar 89 mg %. Glucose tolerance test: normal. Serum proteins: Total protein 7.8 g % and of this, albumin 3.6 g %, alpha-1-globulin 0.7 g %, alpha-2-globulin 1.1 g %, beta-globulin 1.2 g % and gammaglobulin 1.2 g %. The lipoprotein level did not present any abnormal distribution. The glucoprotein level showed a distribution corresponding to that calculated from the protein distribution. No signs were found of special carbohydrate-rich protein fractions apart from those occurring normally.

Course of the disease: During the period of hospitalization, the condition of the infant deteriorated steadily. A constant nasal discharge was present and occasionally pneumonia. An increasing tendency to dyspnoea and cyanosis developed in particular during feeds. The infant was admitted to Queen Louise's Hospital for Children for cardiac catheterization and angiocardiography but was in such poor condition and suffered from respiratory infection that such an investigation was not considered advisable and, for the same reason, adrenaline tolerance test was not

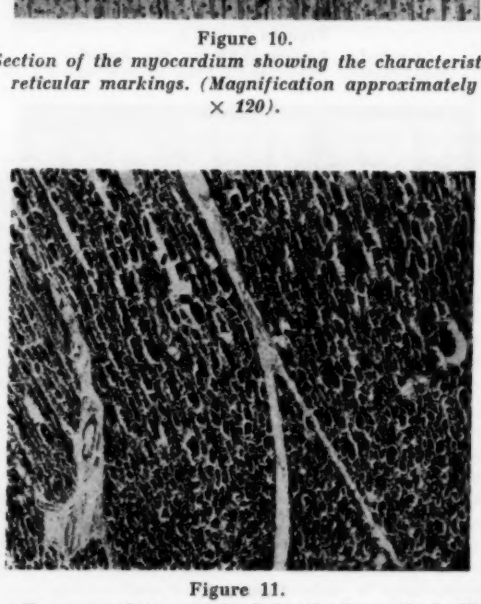


Figure 11.
Cardiac musculature stained by Best's method. The apertures in the reticulum are filled with glycogen. (Magnification approximately × 120).

undertaken. The child was returned to The Paediatric Department on March 4, 1959 where he died the next day at the age of just over 4½ months.

At autopsy, a massive heart measuring 7 × 7 × 6 cm and weighing 125 g was found (normal 32—34 g). The liver weighed 210 g, *i.e.* only slightly more than normal (200 g). The kidneys were normal in size and weighed 20 g apiece and the spleen was slightly smaller than normal and weighed 12 g (normal 17 g). The pancreas and the intestinal canal were of normal appearence. There were areas of atelectasis and bronchopneumonia in the left lung while the right lung showed no atelectasis but had slight bronchopneumonia in the upper lobe. (Normal values according to Watson & Lowrey (15)).

Pathological examinations: (A. Søeborg Ohlsen, M.D., Senior Pathologist): Biopsy of striated muscle: One section stained with haematoxylin-eosin and by van Gieson's method showed severe changes. Instead of the normal pattern, marked vacuolization was found. Fibrils with striation could only be observed near the sarcolemma (Figure 7).

In a section from the specimen fixed in absolute alcohol and stained with Best's carmine, red masses were found at the sites of the vacuoles indicating that glycogen accumulations were concerned. (Figure 8).

Autopsy: On opening the *heart,* no endocardial changes were found. There was no septum defect and no patent foramen ovale.

The wall of the right ventricle measured about 10 mm and that of the left ventricle about 20 mm. The coronary arteries originated in the normal manner and were of normal type.

The myocardium was reddish-yellow and very firm but the fixation in absolute alcohol was probably responsible for this. The fibrils of the myocardium were broader than normal. The structure of the fibrils was interrupted in a singular manner by greater and lesser vacoules. The striation of the muscle fibres was retained where these were intact. The nuclei were somewhat larger than normal. On staining with Best's carmine an intensive and frequently nodular red staining was seen corresponding to the vacuolization. (Figures 10 and 11). This indicates the massive glycogen content.

A specimen from the *striated musculature* showed more marked vacuolization. The vacuoles were of varying size. The distribution of the nuclei of the sarcolemma appeared to be normal. On staining with Best's carmine, intensive red staining corresponding to the vacuolization was found. The stained materiel was nodular. Thus massive accumulation of glycogen had taken place.

The specimen of *liver* tissue consisted of polygonal liver cells which were frequently slightly larger than normal. The cytoplasm showed vacuolization from slight to varying extents. The finely formed round nuclei were situated centrally. The Kupffer cells were of normal type. No infiltrative processes were observed in the portobiliary spaces. The tissue showed local evidence of stasis. On staining with Best's carmine, intensive red staining was observed corresponding to the vacuolization. Thus, considerable accumulation of glycogen had occurred.

In the specimens of *pancreatic* tissue, serous composite alveolar gland cells and their ducts were observed. Isolated granular acinus cells were slightly vacuolated.

The islets of Langerhans were found between the acini. The beta cells were somewhat vacuolized. The islets of Langerhans were rather large. On staining with Best's carmine, quite pronounced red staining of the islets of Langerhans was found corresponding to the vacuolization found here. Red granules were also observed in isolated acinus cells.

In the specimen of tissue from the *spleen,* slightly distended pulp and venous sinusoids were seen. The cells of the pulp had a slightly hyperplastic appearance. Isolated pulp cells had vacuolized cytoplasm. The lymphatic tissue component was nearly normally represented. The arterioles were of normal type. On staining with Best's carmine, red stained granules were observed in several pulp cells.

In the specimen from the *kidney,* glomeruli of normal structure were observed. The cells forming the proximal convoluted tubules had slightly vacuolized cytoplasm. Considerable vacuolization was also observed in the collecting tubules. On staining with Best's carmine, intensive staining was observed in the convoluted tubules and in the collecting tubules.

DISCUSSION

This case of generalized glycogenosis presented all the characteristic features described for this disease so that the diagnosis could be established with considerable certainty on a purely clinical basis and, finally, it could be confirmed by biopsy of muscle tissue.

The investigations undertaken in this case do not contribute anything new to the present knowledge of the condition. It should, however, be noted that the serum transaminase determination showed a considerably raised GO-transaminase value of 372 units (normal: < 40). Such a result might well be anticipated but, as far as the author has been able to ascertain, this investigation has not be conducted in cases of this disease previously reported.

Finally, it is of particular interest that this is the first case of the condition to be described from Scandinavia.

SUMMARY

The clinical picture of generalized glycogenosis (glycogen storage disease of the heart) is reviewed on the basis of the most recent literature.

A typical case of generalized glycogenosis in a boy aged 3½ months is reported. The infant was the second child of related parents. The infant was healthy and thrived until the age of six weeks. Thereafter, dyspepsia occurred associated with increasing weakness, and gradually cardiac symptoms also made their appearance.

Investigations revealed a globular enlarged heart, typical ECG, marked macroglossia, hypotonia of the muscles which were pseudohypertrophic. Biopsy from a striated muscle confirmed the diagnosis.

The laboratory findings were normal apart from the considerably raised serum transaminase (GO-transaminase) and moderately raised serum

potassium and slightly lowered sodium and chloride values. The infant died a month later from cardiac and respiratory failure. The autopsy findings were characteristic. The case appears to be the first to be published from Scandinavia.

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SMOKING HABITS IN TWINS

By ELISABETH RAASCHOU-NIELSEN

The observed association of lung cancer and cigarette smoking has been interpreted in more than one way.

Several authors consider cigarette smoking a cause of lung cancer (1, 2, 5, 6), while others, above all Fisher (3), have put forward the hypothesis, that cigarette smoking and cancer of the lung both have a common cause, a special constitutional make up. The latter has reported smoking habits of 51 monozygotic and 31 dizygotic German male twins. A greater concordance was found within the monozygotic twin pairs than within the dizygotic pairs. The same author has published data concerning the smoking habits in English girl twins. The concordance rate in a group of 27 monozygotic twin pairs, brought up apart, was as high as in a comparable, but nonseparated monozygotic group.

Friberg et al. (4) have demonstrated significantly greater concordance concerning smoking habits within 59 pairs of adult monozygotic twins than within 59 dizygotic pairs.

The purpose of the following investigation is to elucidate in a large material of unselected twins the question of the influence of hereditary factors on smoking habits.

From the Institute of Human Genetics, University of Copenhagen. (Director: Professor *Tage Kemp*).
The statistical calculations were carried out by *A. Nielsen*, lic. act.

The research reported herein was performed pursuant to a contract with the U. S. Public Health Service, Department of Health, Education and Welfare.

MATERIAL

At the University Institute of Human Genetics, Copenhagen, all twins born in Denmark during the years 1870—1910 are at present being collected from the registrations of births. Till now 17,600 pairs have been compiled and of these 4,700 pairs have been completely investigated (9, 10).

Of this initial material of 4,700 twin pairs the following groups have not been included in this present material:

1. All different-sexed pairs of dizygous twins.
2. All pairs of twins, one or both of whom are dead, are permanent patients in a mental institution, or are living abroad.
3. All pairs of twins, one or both of whom have previously refused to cooperate in an investigation or have been unable to furnish pertinent information.

After the omission of these groups 1240 pairs of twins were left for this investigation.

Table 1.
Survey of the Material.

Number of pairs, to whom questionnaires have been sent	1240
Number of pairs, of whom both partners have answered the questionnaire	862
Number of pairs, of whom one or both have not answered in spite of repeated applications	341
Not fit for analysis (not cooperative, questionnaires poorly completed, questionable zygosity)	37
Written answers from both partners	862
Verbal information from one or both partners	32
Total	894

The distribution of the 894 pairs was investigated according to sex, age, zygosity and place of residence, and the sample was not found to be inhomogenous as to the factors mentioned.

INVESTIGATION

Information on zygosity and diseases of the twins originate from the initial investigation (9, 10). A questionnaire was sent to each of the twins with inquiries concerning the following data of interest for the present investigation:

All admissions to hospital (where, when, why?)

Have you suffered from: asthma, chronic bronchitis, peptic ulcer, nervous or mental disorders, or diseases of the heart?

Do you look much like your twin?

Have you been confused by your parents?

By acquaintances?

For the collection of information on smoking habits the following questionnaire has been used:

1. Do you smoke?
   yes ..... no .....
2. How much do you usually smoke a week?
   cigarettes ..... a week
   cigarillos ..... a week
   cigars ..... a week
   pipe tobacco .. a week
   Do you use chewing tobacco ..... a week
3. When did you start smoking regularly?(every day) age .... or year ....
4. Do you inhale? yes .... no ....
5. Have you cut down the amount of tobacco or have you stopped smoking after the beginning of the discussion of the importance of tobacco smoking for cancer of the lung? yes .... no ....
6. Did you change the amount of tobacco used for other reasons? yes .... no ....
7. What was previously your average amount of tobacco used? (this question is answered only if you answered question 5 or 6 in the affirmative.)

This questionnaire resembles the one used in "The Danish National Morbidity Survey of 1950" (7, 8).

Before any result could be estimated, three questions had to be answered:

1. How is the accuracy of replies to questionnaires on zygosity?
2. How is the accuracy of replies to questionnaires on smoking habits?
3. Are the respondents in this smoking investigation representative of all twins asked?

The Problem of Zygosity.

A sample of twins was examined according to 7 blood group systems ($A_1 A_2$ BO, MNS, Rhesus (with five sera), P, Lewis, Kell and Duffy) and 1 serum group system (Gamma) in order to elucidate the zygosity diagnosis. 119 pairs, some of these taken out by chance and some because of certain diseases, have been investigated in this way.

62 pairs considered themselves monozygous:
- 60 pairs were identical as to the blood groups mentioned.
- 2 pairs were not identical.

57 pairs considered themselves dizygous:
- 53 had different blood groups.
- 4 pairs were identical as to the blood groups mentioned.

By use of the 7 blood group systems and 1 serum group system 96 % of all pairs of dizygous twins from the ordinary Danish population may be placed in the correct group, *i.e.*, 4 % may have identical blood groups (11).

The accordance between the statement of the twins concerning their zygosity and the results of the blood tests are such, that it was felt justified to make the diagnosis of zygosity in the remaining part of the material on the basis of the questionnaires.

The Reliability of Replies Concerning Smoking Habits.

To check the accuracy of the information on smoking habits, won by questionnaires, a subsample of twins (50 pairs, 25 monozygotic and 25 dizygotic) has been taken out by chance and personally interviewed.

4 pairs were not cooperative.

46 pairs = 92 persons have been interviewed.

The result is shown in Table 2.

Table 2.

A Comparison Between Grouping of Persons According to Questionnaires and as Controlled by Personal Interviews with the same Persons.

	By questionnaire	By personal interview
Non-smokers	29	→ 29
occasional smokers	10	(7) → 7
		(3) ↓
former smokers	10	→ 13
regular smokers	35	(29) → 29
		(6) ↓
heavy smokers	8	→ 14

Each person is classified as a nonsmoker, an occasional smoker, a former smoker, a regular smoker or a heavy smoker.

Nonsmokers: such persons, as have denied smoking on the questionnaire without further comment.

Occasional smokers: persons, who smoke less than 1 cigarette, 1 cigarillos, 1 cigar or 1 pipeful a day.

Table 3.

Number of Smokers Observed in Various Groups of the Twin Series Compared with the Number that would have been Expected in Groups of Equal Number of Unselected Persons of the same Age, Sex and Residence, in View of the Percentage of Smokers Found in the Danish Population 1952—53.

Smokers: All who Admit to Smoking at all.
Age group I: Born 1870—1890; II: Born 1891—1900; III: Born 1901—1910.

			Males			Females	
			Total number	Number of smokers observed	Number of smokers expected	Total number	Number of smokers observed	Number of smokers expected
Copenhagen	Dizygotic	I	8	5	5,2	26	15	6,9
		II	34	29	26,7	36	18	11,5
		III	50	44	41,2	96	52	43,8
		Total	92	78	73,1	158	85	62,2
	Monozygotic	I	12	7	7,8	16	5	4,3
		II	28	24	18,9	34	22	10,9
		III	40	34	33,0	56	33	25,5
		Total	80	65	59,7	106	60	40,7
The Provinces	Dizygotic	I	48	23	27,7	52	6	2,1
		II	82	53	56,1	124	26	18,2
		III	162	117	126,3	218	64	56,2
		Total	292	193	210,1	394	96	76,5
	Monozygotic	I	28	14	16,2	48	2	1,9
		II	70	53	46,7	84	29	12,3
		III	88	60	68,6	102	25	26,3
		Total	186	127	131,5	234	56	40,5

Former smokers: nonsmokers or occasional smokers, who once were regular smokers.

Regular smokers: persons, who smoke 1 or more cigarettes (cigars, cigarillos, pipefuls) daily.

Heavy smokers: are regular smokers, whose consumption is:

15 or more cigarettes daily (defined according to Danish smoking habits, cf. the criteria in U.S., where only about half a cigarette is consumed (7).

1 cigarette = 1 g of tobacco.

4 or more cigars daily. 1 cigar = 7 g of tobacco.

7 or more cigarillos daily. 1 cigarillo = 4 g of tobacco.

25 g pipe tobacco or more daily.

Mixed smokers are classified according to their total consumption.

The group nonsmokers is identical by the two procedures. 3 occasional smokers were reclassi-

Table 4.

Distribution of Concordant and Discordant Pairs (in Respect of Amount of Tobacco Smoked) in Dizygotic and Monozygotic Twins. Per Cent of Total Number.

I: Nonsmokers.
II: Occasional smokers.
III: Former smokers.
IV: Regular smokers. } Considered one group in calculating
V: Heavy smokers. } the concordance rate.

Males. Dizygotic pairs. Total: 223

Twin I \ Twin II	I	II	III	IV	V
I	5.8				
II	2.7	0.0			
III	3.1	0.9	1.3		
IV	13.0	2.7	13.0	27.4	
V	3.1	0.4	2.2	19.3	4.9

99.8

Males. Monozygotic pairs. Total: 147

Twin I \ Twin II	I	II	III	IV	V
I	9.5				
II	2.7	0.7			
III	3.4	0.7	4.1		
IV	6.1	2.7	10.9	28.6	
V	0.0	0.0	1.4	15.0	14.3

100.1

Females. Dizygotic pairs. Total: 328

Twin I \ Twin II	I	II	III	IV	V
I	46.0				
II	9.8	1.2			
III	3.7	0.6	0.3		
IV	18.0	7.0	1.5	7.9	
V	1.2	0.3	0.0	2.1	0.3

99.9

Females. Monozygotic pairs. Total: 196

Twin I \ Twin II	I	II	III	IV	V
I	46.9				
II	7.7	2.6			
III	2.6	0.0	0.5		
IV	11.2	3.1	1.5	19.4	
V	2.0	0.0	0.0	2.6	0.0

100.1

Table 5.

Comparison of the Frequency of Concordance in Monozygotic and Dizygotic Twins.

	Females			Males		
	MZ conc/total	DZ conc/total	X^2 D.f.	MZ conc/total	DZ conc/total	X^2 D.f.
Year of birth						
1870—1890	31/34	33/46	3.48 1	15/22	15/30	1.05 1
1891—1900	45/71	60/97	0.00 1	37/55	36/67	1.78 1
1901—1910	65/91	97/185	11.79**3			6.10 3
Sum			8.31**1	54/70	80/126	3.27 1
Residence						
The Provinces	87/117	130/197	2.03 1	63/93	81/146	3.07 1
The Prov.+Coph.	19/26	19/52	7.89**1	11/14	16/31	1.89 1
Copenhagen	35/53	41/79	2.05 1	32/40	34/46	0.17 1
Sum			11.97**3			5.13 3

* means significance with regard to the 5 %-level.
** means significance with regard to the 1 %-level.

Table 6.

Distribution of Concordant and Discordant Pairs in Respect of Type of Tobacco Smoked. Mixed Smokers are Classified According to the Greatest Consumption.

43 pairs of monozygotic female twins, in which both partners are regular smokers:

Twin I \ Twin II	Cigarettes	Cigarillos Cigars
Cigarettes	12	
Cigarillos Cigars	8	23

34 pairs of dizygotic female twins, in which both partners are regular smokers:

Twin I \ Twin II	Cigarettes	Cigarillos Cigars
Cigarettes	14	
Cigarillos Cigars	9	11

85 pairs of monozygotic male twins, in which both partners are regular smokers:

Twin I \ Twin II	Cigarettes	Cigarillos Cigars	Pipe
Cigarettes	13		
Cigarillos Cigars	12	18	
Pipe	7	11	24

115 pairs of dizygotic male twins, in which both partners are regular smokers:

Twin I \ Twin II	Cigarettes	Cigarillos Cigars	Pipe
Cigarettes	8		
Cigarillos Cigars	15	24	
Pipe	17	30	21

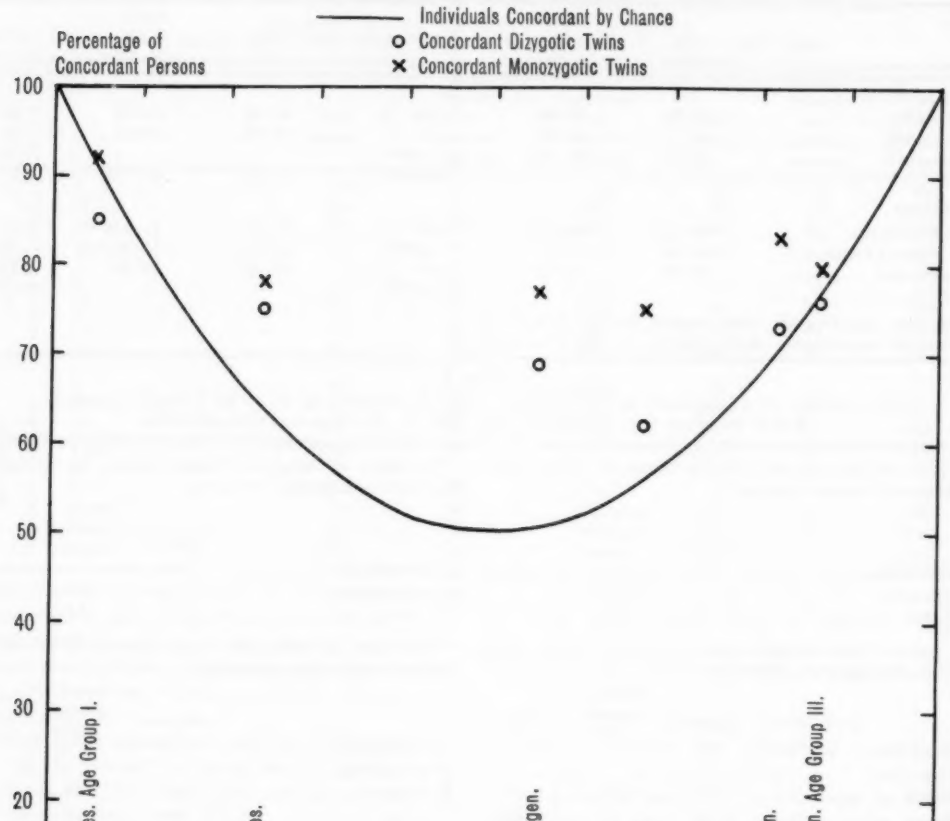
Table 7.

Incidence of Various Diseases in Smoking and Not Smoking Twins, Given in Number of Affected Individuals.

1: Both partners nonsmokers
2: Both partners smokers (definition, see text)
3: One partner smoker, the other nonsmoker.

		MZ		DZ	
		Concordant	Discordant	Concordant	Discordant
Asthma and chronic bronchitis	1.	2	10		21
	2.	6	3	4	9
	3. A. Smoker presents sympt.		2		4
	3. B. Nonsmoker " "				4
Neuroses	1.	6	4	8	14
	2.	6	8	4	6
	3. A.				6
	3. B.		1		8
Peptic ulcer	1.		8		12
	2.		13	2	12
	3. A.		2	2	4
	3. B.				2
Coronary occlusion	1.		1		1
	2.				1
	3. A.				
	3. B.				2
Cancer of the Lung		No cases			

Fig. I.
Concordant Pairs in a Group as Compared to the Percentage of Smokers in the Same Group. Smokers: Definition see text.



fied as former smokers and 6 regular smokers were reclassified as heavy smokers. No regular smokers were classified as nonsmokers or occasional smokers. A good correspondance was consequently found between the information obtained by questionnaire and by personal visit.

As to the kind of tobacco used there is a good correspondance as well. The preferred form of smoking was always correctly written on the questionnaire. 14 smokers proved to have minor deviations in their statements concerning the less frequently used forms of tobacco.

The Composition of the Respondents.

The problem whether the respondents were representative of all twins was investigated. A subsample of non-respondents was personnally interviewed, and further the smoking habits of the total twin series were compared (Table 3) with those in the Danish population, found by Hamtoft & Lindhardt 1952—53 (7, 8).

In the Copenhagen area 87 persons had not answered the questionnaire. Attempts were made to interview 45 of these personnally. 5 persons were not cooperative and 3 persons were dead. 37 individuals were interviewed and distributed according to smoking habits:

Nonsmokers	6
Occasional smokers	1
Former smokers	7
Regular smokers	12
Heavy smokers	11

It is seen from Table 3 that the number of male smokers is in accordance with the number found in the investigation of 1952—53. But as to female smokers there is a significantly greater number in this twin series, compared with the findings in the investigation referred to. The greater num-

ber of smoking females is most conspicuous in the oldest age group in Copenhagen and in the medium age group in the provinces. Undoubtedly the explanation is that smoking has become more common among women during the past 7 years. Thus the twins investigated reflect the smoking habits of the Danish population at present.

RESULTS

The results are shown in Table 4—7 and Fig. 1.

Comparisons have been made between the monozygotic and dizygotic twins as to the amount (Table 4 and 5) and to the type (Table 6) of tobacco smoked.

Further a comparison has been made as to smoking in any form (Fig. 1).

In Table 4 the frequency of corcordance and discordance was examined for each sex. Concordant are considered all pairs placed in the diagonals and pairs, in which one partner is a regular smoker and the other a heavy smoker. In each group of age it was tested by means of a χ^2-value with 1 degree of freedom, if the probability of concordance and discordance could be the same. The result is shown in Table 5.

It is seen that by distributing the material according to age, there is a significant difference in one female group, but in no male group. The frequency of concordance in all groups being greatest for monozygotic twins, the χ^2-values have been added, by means of which significance again is found in women, but not in men. These two χ^2-values total 17.89 with 6 degrees of freedom, and this value is significant with regard to the 1 %-level.

By the distribution according to residence there is a significant difference for females too, but not for men. The total χ^2-value shows significance as well.

As was just demonstrated, each group ought to have been divided according to age, the age being important for the frequency of concordance. However the result of such a division should only have been, that the numbers in the various groups would have been very small.

The subsamples of personnally interviewed twins, and twins with the zygosity confirmed by blood test were investigated separately according to amount of tobacco used. They showed the same pattern as all Copenhagen twins.

A greater concordance in monozygotic twins is found too as to the type of tobacco used. Concordant pairs are those found in the diagonals of the tables (6).

The concordance in respect of smoking or not smoking was investigated. In this analysis occasional smokers were classified as smokers, and former smokers as nonsmokers. In Fig. I the rate of concordance within subgroups of the twin series is shown in relation to the percentage of smokers within the group in question. It is seen that the rate of concordance in dizygotic twins nearly always is higher than that which might be expected by chance.

The incidence of various diseases in smoking and non smoking twins is shown in Table 7. Here occasional smokers are classified as nonsmokers and former smokers are not included. No case of cancer of the lung is seen, all twins in this series being still alive.

DISCUSSION

Concerning the amount of tobacco smoked a significantly greater concordance is found within monozygotic twins than within dizygotic twins for the series as a whole. The greater concordance in monozygotic twin pairs is also found in respect of type of tobacco used and in respect of smoking in any form.

It is seen that age, sex, and residence are factors which influence the rate of concordance. The frequency of tobacco smoking in a group will affect the number of concordant twins. The percentage of concordant dizygotic twins is higher than what might be expected by chance, and this too indicates environments to be important for the concordance rate.

Thus both genetic and environmental factors play a part in forming the smoking habits of the individual. A further investigation of the discordant monozygotic twins might perhaps throw more light on the psychology of smoking.

It has been pointed out, that what here is interpreted as genetic factors, really are environmental, due to a more uniform environment of the monozygotic twins. The findings in a series of separated twins do not agree with this. In 12 monozygotic pairs of twins (born 1880—1934), brought up apart, Juel-Nielsen (12) has found the same rate of concordance concerning smoking habits as is seen in the monozygotic pairs in this series. As to amount of tobacco smoked, 75 per cent of the separated twins are concordant, compared with 69 per cent concordant pairs in this series as a whole.

Attempts have been made to investigate a possible constitutional association between regular smoking and certain diseases. The number of affected twins are too small to permit any conclusion. The morbidity in monozygotic twins who are discordant concerning smoking habits would have been of interest in this respect. Important would also have been the finding in monozygotic and dizygotic smoking twins.

SUMMARY

In an unselected series of 894 pairs of twins a significantly greater concordance concerning smoking habits is found within the monozygotic twins than within the dizygotic pairs.

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THE THEORETICAL BASIS AND PRACTICAL EMPLOYMENT OF IMMUNO-ELECTROPHORESIS

WITH SPECIAL REGARD TO SERUM PROTEINS

By JØRGEN CLAUSEN

SUMMARY OF THESIS

The thesis is a survey meaut to elucidate the theory and practical employment of immuno-electrophoresis, both as a diagnostic tool and as an instrument to continued exploration of proteins and their pathology.

The theoretical basis of immuno-electrophoresis, to combine an electrophoretical separation of a mixture of proteins with a subsequent antigen-antibody reaction is elucidated by discussing the electrophoretical migration and passive diffusion of proteins in an agar gel. It is pointed out that immuno-electrophoresis can visualize more than twenty different precipitation bows corresponding to more than twenty different specifically reacting antigens (protein fractions) in human and mouse serum. The identification and characterisation of these fractions can be performed by combination of the immuno-electrophoresis with other methods, such as absorption experiments, Ouchterlony diffusion (developed as the combined diffusion method), acid electrophoresis, staining for oxidase activity, carbohydrate- and lipoprotein content, and auto-radiography, as well as by means of classical physico-chemical procedures on the isolated serum proteins, using immuno-electrophoresis as the tool of choice to control the purity of the preparation.

In this way it has been possible to identify and characterize 16 of the above mentioned serum fractions among which three (beta-2-A, beta-2-M and gamma) have the character of antibodies, for which reason they have been designated immunoglobulins.

By autoradiography combined with immuno-electrophoresis it could be demonstrated that under normal circumstances the thyroid hormone is transported by the three lipoproteins in serum.

On the basis of the normal immuno-electrophoretical tracings the protein pathology of man and mouse is described concerning paraproteinemia, chronic stimulation (chronic infections and autoimmunisation), nephrosis, amyloidosis, and hepatomas in mice.

In paraproteinemia (myelomatosis and macroglobulinemia) the immunoglobulins are affected in a specific way, *i. e.*, a paraprotein (which can be of beta-2-A, gamma- or beta-2-M type) in serum will very often be associated with a hypo-immunoglobulinemia of all the immunoglobulins which do not have paraprotein character.

In this way myelomatosis in man as well as plasma cell leukemia in mice can be divided into the two main groups: 1) *Beta-2-A myeloma* associated with a hypo-gamma as well as a hypo-beta-2-M globulinemia. (This type is not with certainty distinguished in mice. 2) *Gamma myeloma* with gamma paraprotein of 1) beta-2-, 2) M- (intermediate-) and 3) slow gamma mobility all of which is associated with a hypo-beta-2-A globulinemia, a hypo-gamma globulinemia in non affected areas as well as a hypo-beta-2-M globulinemia.

Macroglobulinemia (which is also described in mice) is characterized by an increase in beta-2-M associated with a decrease in beta-2-A.

Nephrosis is characterized by a heavy increase in alpha-2-macroglobulin, alpha-2-lipoprotein, alpha-2-haptoglobin, as well as in alpha-1-glycoprotein associated with a decrease in albumin and transferrin in serum. Immuno-electrophoresis of concentrated urine reveal the existence in this disease of all serum proteins, among which especiall the transferrin content should be noticed because this will explain the hypotransferrinemia giving a delayed iron metabolism and causing hypochromic anemia.

Amyloidosis does not seem to show specific serum protein changes. On the other hand, in the pyroninophilic-proliferative phase revealed by histological examination (T e i l u m) a hyper-immunoglobulinemia is evident. In the intermediate retarding exhausted phase in the RED system the serum proteins change mobility and concentration in the alpha-1-area. In the phase with amyloidotic development in the kidneys a nephrotic syndrome is developed.

Finally in mice with primary hepatomas immuno-electrophoresis showed an increase in transferrin. The significance of this is discussed.

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